have been amended to provide proper antecedent basis in each claim. It should be noted that base claim 24 is a method for the *site-specific* recombination of DNA.

Rejection Under 35 U.S.C. § 112, second paragraph

Claims 1-49 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite (claim 2 was examined and the term "compatibility sequences" assumed to be comprised in the recombination sites). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Examiner states that it is unclear that the "mutant recombinase" produces the stated recombination between the substrate sites. The Examiner is assuming that the mutant recombinase of section "(a)" acted on the substrate to produce a recombination event. The Applicants respectfully remind the Examiner that claim 1 is directed to, *inter alia*, "determining **if** recombination occurs between the first and second recombination sites, and determining **if** recombination occurs between the third and fourth recombination sites..." (emphasis added). *It is not known whether the mutant recombinase of section "(a)" acts on the substrate to produce a recombination event until a determination has been made in step "(b)".* Once a determination has been made (via assaying reporter gene activity), only then can one definitively assert that the variant recombinase *mediates* recombination. The Applicants submit that "bringing into contact" the recombinase and the two nucleic acid sequences is all that is required because recognition and mediation of recombination are inherent characteristics to the recombinase (and will occur if these characteristics are "proper" for the sites).

The Examiner has asserted that there is no definition provided in the claims or specification for the term "compatibility sequences". However, as stated at page 5, lines 21-24, compatibility sequences are in the recombination site (other than the sequences required for recognition of the site by the recombinase), that must be similar in a pair of recombination sites for recombination to occur between them. It is important to note that recombination sites comprise *recognition sequences and compatibility sequences.*

Claim 3 has been amended to refer to recombination between sites 1 and 2 as being significantly reduced. Applicants submit that the proper test is whether or not one skilled in the art could determine specific values for a "significant" reduction in recombination (in the present case) based upon the disclosure. See *In re Mattison*, 509 F.2d 563, 184 USPQ 484 (CCPA 1975). The use of reporter gene constructs are well known in the art. One of ordinary skill in the art would have no problem recognizing a "significant" reduction in recombination, as assayed by the disclosed reporter gene constructs/assays and/or gel electrophoresis of fragment obtained from recombination events. One of ordinary skill in the art will additionally be able to correlate the level of reporter gene activity, or level of brightness of Ethidium-Bromide stained DNA fragments on agarose gels, with recombination frequency (see, for example, lines 28-13, bridging pages 93 and 94).

Additionally, the analogous phrase "an effective amount" was held to be definite where the amount was not critical and those skilled in the art would be able to determine from the written disclosure, including the Examples, what an effective amount is. *In re Halleck*, 422 F.2d 911, 164 USPQ 647 (CCPA 1970).

Claim 24 has been amended to refer to a set of recombination sites using different denotations (i.e. fifth site, sixth site). These sites are recognized and recombined by the variant recombinase *identified* in claim 1. The sites may or may not comprise identical sequences used to identify the variant recombinase in claim 1 (i.e. the first, second, third and fourth sites). All claims dependent from the newly amended claim 24 have been amended to provide proper antecedent basis in each claim.

Claim 30 now finds proper antecedent basis in claims 24 and 29.

Rejection Under 35 U.S.C. § 102

Claims 1-4, 21, 24-25, 33-35, 40 and 43 were rejected under 35 U.S.C. § 102(b) as being anticipated by *Gene* (1994) 34:197-206 by McCormick *et al.* ("McCormick"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Examiner has asserted that McCormick teaches a mutant recombinase. McCormick is directed to constructing a mutant of Tn3 (Tn3 #2), wherein the resulting mutant harbors an additional 63 bp near the amino-terminal coding region of tnpA. The gene, tnpA, encodes a *transposase*. Tn3 codes for (i) a transposase, (ii) a repressor (tnpR), or "resolvase", and (iii) β-Lactamase (see page 197 of McCormick). It should be recognized that a transposase is not a recombinase. As shown in the accompanying pages from "Molecular Genetics of Bacteria" by Larry Snyder and Wendy Champness ("Snyder") 1997), if the transposase were intact (note that it is still functional in the Tn3 #2 mutant of McCormick), it would make single-stranded breaks at the junction between the transposon and the donor DNA (pMB of McCormick) and a double stranded break in the target DNA (plasmid HS1 of McCormick). The ends of the target DNA are

then ligated to the ends of the transposon (see Figure 8.10 of page 204 of Snyder), thereby forming the co-integrate. Therefore, the *formation* of the co-integrate would *not* result from *recombination*. However, McCormick teaches that the transposase is non-functional, thereby somehow driving a another process that results in the co-integrate. The co-integrate is resolved (*by the resolvase*) via recombination between two res sites in the co-integrate (the second res site is copied from the original in the Tn3 element). As taught in McCormick, the mutation (a 63 bp insert *in the amino terminal coding region of tnpA*) is not in the resolvase (the only recombinase taught in McCormick). Therefore, McCormick does not teach a *mutant recombinase*. McCormick does not even suggest a mutation in the resolvase, or *any* recombinase.

In addition, McCormick fails to teach any alteration of *substrate specificity* with regard to a mutant recombinase, as compared to a wild type recombinase. As clearly stated at page 4 of the present application, "the constructs contain variant recombination sites that *are not recognized by non-mutant recombinase* but will undergo recombination in the presence of a mutant recombinase with altered specificity" (emphasis added; see lines 5-8). McCormick fails to mention any variation with regard to recombinase specificity.

Claims 1-4, 23-25, and 48 were rejected under 35 U.S.C. § 102(b) as being anticipated by *Biochem. Biophys. Res. Comm.* 253:588-593 (1998) by Lee *et al.* ("Lee"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The present claims are directed to identifying *variant recombinases* that mediate recombination at variant sites and producing site-specific recombination of DNA comprising, *inter alia*, contacting a *variant recombinase* with DNA sequences. Applicants respectfully

submit that Lee does not teach a variant recombinase. Lee is directed to *modifying DNA sites* that may be utilized by the wild type Cre protein (see Materials and Methods). A variant recombinase is a recombinase that has been mutagenized or altered in some way (see present specification at page 26, lines 25-27). The Applicants submit that the Lee only teaches the use of wild type Cre and its recognition of the sequences provided in the *in vitro* assays. Lee only eludes to the possible use of a modified Cre that might bind to candidate target sequences (see page 590, second column), without any reference to how one might go about producing such a protein.

Rejection Under 35 U.S.C. § 103

Claims 1-49 were rejected under 35 U.S.C. § 103(a) as being unpatentable over , in view of *Gene* (1994) 34:197-206 by McCormick *et. al.* ("McCormick") or *Biochem. Biophys. Res. Comm.* 253:588-593 (1998) by Lee *et. al.* ("Lee") in view of U.S. Patent No. 5,677,177 to Wahl *et. al.* ("Wahl"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

McCormick and Lee

As discussed in the foregoing sections under 35 U.S.C. § 102, neither McCormick or Lee teach or suggest a variant recombinase. Additionally, neither McCormick or Lee teach the alteration of substrate specificity of a variant recombinase as compared to those sequences recognized by the wild type recombinase.

Wahl

Wahl fails to teach a variant recombinase. Wahl fails to teach any alteration of substrate specificity.

Summary

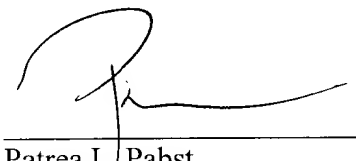
None of the cited prior art teach a variant recombinase, wherein the "variant" characteristic lies within the specificity of the recombinase. There is no teaching in the prior art, individually or in combination, of a variant recombinase with altered specificity (i.e. recognizing sequences that are not recognized by non-mutant recombinases).

Claim Objections

Claim 37 was objected to under 37 C.F.R. § 1.75(c) as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicants respectfully traverse this objection to the extent that it is applied to the claims as amended.

Allowance of claims 1-49 is respectfully solicited.

Respectfully submitted,



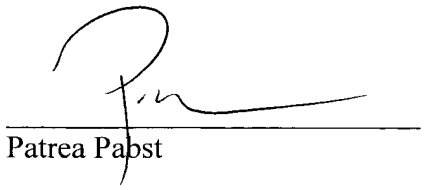
Patrea L. Pabst
Reg. No. 31,284

Date: May 28, 2002

HOLLAND & KNIGHT LLP
One Atlantic Center, Suite 2000
1201 West Peachtree Street
Atlanta, Georgia 30309-3400
(404) 817-8473
(404) 817-8588 (Fax)

Certificate of Mailing Under 37 C.F.R. § 1.8(a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.



Patrea Pabst

Date: May 28, 2002

MARKED UP VERSION OF AMENDMENTS PURSUANT TO 37 C.F.R. § 1.121

Marked Up Version of Amended Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. A method of identifying variant recombinases that mediate recombination at variant recombination sites, the method comprising,

(a) bringing into contact

a mutant recombinase,

a first nucleic acid sequence comprising a first reporter gene and first and second recombination sites, wherein the first and second recombination sites are variant recombination sites, and

a second nucleic acid sequence comprising a second reporter gene and third and fourth recombination sites, wherein the third and fourth recombination sites can be recombined by a non-mutant recombinase,

(b) determining if recombination occurs between the first and second recombination sites, and determining if recombination occurs between the third and fourth recombination sites,

wherein recombination between the first and second recombination sites indicates that the mutant recombinase is a variant recombinase that mediates recombination at variant recombination sites,

wherein recombination between the third and fourth recombination sites indicates that the mutant recombinase retains the ability to mediate recombination at non-variant recombination sites.

2. The method of claim 1 wherein the recombination sites comprise recognition sequences and compatibility sequences,

wherein the recognition sequences of the first and second recombination sites differ from the recognition sequences of the third and fourth recombination sites,

wherein the compatibility sequences of the first and second recombination sites are sufficiently similar to allow recombination between the first and second recombination sites, and

wherein the compatibility sequences of the third and fourth recombination sites are sufficiently similar to allow recombination between the third and fourth recombination sites, and

wherein the compatibility sequences of the first and second recombination sites differ from the compatibility sequences of the third and fourth recombination sites such that neither the first nor the second recombination site can be recombined with either the third or the fourth recombination site.

3. (Amended) The method of claim 1 wherein recombination frequency between the first and second recombination sites [cannot be recombined] mediated by a non-mutant recombinase [to a significant extent] is signficantly reduced.

4. The method of claim 1 or 2 wherein the first and second recombination sites have identical sequences, and wherein the third and fourth recombination sites have identical sequences.

5. The method of claim 1 wherein recombination between the first and second recombination sites alters the expression of the first reporter gene, wherein recombination between the first and second recombination sites is determined by determining if expression of the first reporter gene is altered, and

wherein recombination between the third and fourth recombination sites alters the expression of the second reporter gene, wherein recombination between the third and fourth recombination sites is determined by determining if expression of the second reporter gene is altered.

6. The method of claim 5 wherein recombination between the first and second recombination sites allows the first reporter gene to be expressed.

7. The method of claim 6 wherein the first nucleic acid sequence further comprises a spacer sequence flanked by the first and second recombination sites, wherein the spacer sequence interrupts the first reporter gene such that the first reporter gene is not expressed, wherein

recombination of the first and second recombination sites excises the spacer sequence which allows the first reporter gene to be expressed.

8. The method of claim 6 wherein a portion of the first reporter gene is inverted, wherein the inverted portion of the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites inverts the inverted portion of the first reporter gene which allows the first reporter gene to be expressed.

9. The method of claim 5 wherein recombination between the first and second recombination sites prevents expression of the first reporter gene.

10. The method of claim 9 wherein the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites excises the first reporter gene which prevents expression of the first reporter gene.

11. The method of claim 9 wherein a portion of the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites inverts the flanked portion of the first reporter gene which prevents expression of the first reporter gene.

12. The method of claim 5 wherein recombination between the third and fourth recombination sites allows the second reporter gene to be expressed.

13. The method of claim 12 wherein the second nucleic acid sequence further comprises a spacer sequence flanked by the third and fourth recombination sites, wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene is not expressed, wherein recombination of the third and fourth recombination sites excises the spacer sequence which allows the second reporter gene to be expressed.

14. The method of claim 13 wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene is not transcribed.

15. The method of claim 13 wherein the second reporter gene encodes a protein, wherein the spacer sequence interrupts the second reporter gene such that the protein encoded by the second reporter gene is not translated.

16. The method of claim 13 wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene produces an inactive expression product.

17. The method of claim 12 wherein a portion of the second reporter gene is inverted, wherein the inverted portion of the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites inverts the inverted portion of the second reporter gene which allows the second reporter gene to be expressed.

18. The method of claim 5 wherein recombination between the third and fourth recombination sites prevents expression of the second reporter gene to be expressed.

19. The method of claim 18 wherein the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites excises the second reporter gene which prevents expression of the second reporter gene.

20. The method of claim 18 wherein a portion of the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites inverts the flanked portion of the second reporter gene which prevents expression of the second reporter gene.

21. The method of claim 1 wherein the first nucleic acid sequence is a first nucleic acid construct and the second nucleic acid sequence is on a second nucleic acid construct.

22. The method of claim 21 wherein the first nucleic acid construct is an extrachromosomal vector and the second nucleic acid construct is in the genome of a host cell.

23. The method of claim 1 wherein the first and second nucleic acid constructs are on the same nucleic acid construct.

24. (Amended) A method for producing site-specific recombination of DNA, comprising,

contacting a variant recombinase identified by the method of claim 1 with [first and second] third and fourth DNA sequences,

wherein the [first] third DNA sequence comprises a [first] fifth recombination site and the [second] fourth DNA sequence comprises a [second] sixth recombination site,

wherein the variant recombinase mediates recombination between the [first and second] fifth and sixth recombination sites thereby producing the site specific recombination.

25. (Amended) The method of claim 24 wherein the [first] fifth recombination site, the [second] sixth recombination site, or both, are variant recombination sites.

26. (Amended) The method of claim 24, wherein the [first and second] third and fourth DNA sequences are connected by a pre-selected DNA segment.

27. (Amended) The method of claim 26, wherein the [first and second] fifth and sixth recombination sites have the same orientation and the site-specific recombination of DNA is a deletion of the pre-selected DNA segment.

28. The method of claim 27, wherein the pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

29. (Amended) The method of claim 27 further comprising contacting the variant recombinase with a [fourth] fifth DNA sequence comprising a [third] seventh recombination site, wherein the [second and fourth] fourth and fifth DNA sequences are connected by a second pre-selected DNA segment.

30. (Amended) The method of claim 29 wherein the [first] fifth recombination site is a variant recombination site recognized by the variant recombinase and not by wild type recombinase, and wherein the [second and third] sixth and seventh recombination sites are recombination sites recognized by wild type recombinase and by the variant recombinase.

31. (Amended) The method of claim 30 further comprising, prior to contacting the variant recombinase with the [first, second, and third] fifth, sixth, and seventh recombination sites, contacting the recombination sites with wild type recombinase, thereby producing site specific recombination between the [second and third] sixth and seventh recombination sites resulting in a deletion of the second pre-selected DNA segment.

32. The method of claim 29, wherein the second pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

33. (Amended) The method of claim 26, wherein the [first and second] fifth and sixth recombination sites have opposite orientations and the site-specific recombination is an inversion of the nucleotide sequence of the pre-selected DNA segment.

34. (Amended) The method of claim 33, wherein the [first and second] fifth and sixth recombination sites are variant recombination sites recognized by the variant recombinase.

35. The method of claim 33, wherein the pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

36. (Amended) The method of claim 24, wherein the [second and third] fourth and fifth DNA sequences are introduced into two different DNA molecules and the site-specific recombination is a reciprocal exchange of DNA segments proximate to the recombination sites.

37. (Amended) The method of claim 36, wherein the [first and second] fifth and sixth recombination sites are variant recombination sites recognized by the variant recombinase.

38. (Amended) The method of claim 24 wherein the [second] fourth DNA sequence includes a label, wherein recombination between the [first and second] fifth and sixth recombination sites associates the label with the [first] third DNA sequence.

39. (Amended) The method of claim 38 wherein the [first] third DNA sequence is a large circular DNA molecule.

40. The method of claim 24 wherein recombination occurs in a cell.

41. (Amended) The method of claim 40 wherein the variant recombinase is contacted with the [first and second] third and fourth DNA sequences by introducing into the cell [a third] a sixth DNA sequence comprising DNA encoding the variant recombinase.

42. (Amended) The method of claim 41, wherein the [third] sixth DNA sequence further comprises a regulatory nucleotide sequence and expression of the variant recombinase is produced by activating the regulatory nucleotide sequence.

43. The method of claim 40, wherein the cell is a eukaryotic cell, a mammalian cell, a yeast cell, a fungal cell, a prokaryotic cell, a bacterial cell, an archae bacterial cell, or a cell in a multicellular organism.

44. The method of claim 43 wherein the multicellular organism is a plant, an animal, or a mammal.

45. (Amended) The method of claim 40, wherein the [first and second] third and fourth DNA sequences are connected by a pre-selected DNA segment, wherein the first and second recombination sites have the same orientation and the site-specific recombination of DNA is a deletion of the pre-selected DNA segment.

Clean Version of Amended Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. A method of identifying variant recombinases that mediate recombination at variant recombination sites, the method comprising,

(a) bringing into contact

a mutant recombinase,

a first nucleic acid sequence comprising a first reporter gene and first and second recombination sites, wherein the first and second recombination sites are variant recombination sites, and

a second nucleic acid sequence comprising a second reporter gene and third and fourth recombination sites, wherein the third and fourth recombination sites can be recombined by a non-mutant recombinase,

(b) determining if recombination occurs between the first and second recombination sites, and determining if recombination occurs between the third and fourth recombination sites,

wherein recombination between the first and second recombination sites indicates that the mutant recombinase is a variant recombinase that mediates recombination at variant recombination sites,

wherein recombination between the third and fourth recombination sites indicates that the mutant recombinase retains the ability to mediate recombination at non-variant recombination sites.

2. The method of claim 1 wherein the recombination sites comprise recognition sequences and compatibility sequences,

wherein the recognition sequences of the first and second recombination sites differ from the recognition sequences of the third and fourth recombination sites,

wherein the compatibility sequences of the first and second recombination sites are sufficiently similar to allow recombination between the first and second recombination sites, and

wherein the compatibility sequences of the third and fourth recombination sites are sufficiently similar to allow recombination between the third and fourth recombination sites, and

wherein the compatibility sequences of the first and second recombination sites differ from the compatibility sequences of the third and fourth recombination sites such that neither the first nor the second recombination site can be recombined with either the third or the fourth recombination site.

C1 3. (Amended) The method of claim 1 wherein recombination frequency between the first and second recombination sites mediated by a non-mutant recombinase is significantly reduced.

4. The method of claim 1 or 2 wherein the first and second recombination sites have identical sequences, and wherein the third and fourth recombination sites have identical sequences.

5. The method of claim 1 wherein recombination between the first and second recombination sites alters the expression of the first reporter gene, wherein recombination between the first and second recombination sites is determined by determining if expression of the first reporter gene is altered, and

wherein recombination between the third and fourth recombination sites alters the expression of the second reporter gene, wherein recombination between the third and fourth recombination sites is determined by determining if expression of the second reporter gene is altered.

6. The method of claim 5 wherein recombination between the first and second recombination sites allows the first reporter gene to be expressed.

7. The method of claim 6 wherein the first nucleic acid sequence further comprises a spacer sequence flanked by the first and second recombination sites, wherein the spacer sequence interrupts the first reporter gene such that the first reporter gene is not expressed, wherein

recombination of the first and second recombination sites excises the spacer sequence which allows the first reporter gene to be expressed.

8. The method of claim 6 wherein a portion of the first reporter gene is inverted, wherein the inverted portion of the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites inverts the inverted portion of the first reporter gene which allows the first reporter gene to be expressed.

9. The method of claim 5 wherein recombination between the first and second recombination sites prevents expression of the first reporter gene.

10. The method of claim 9 wherein the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites excises the first reporter gene which prevents expression of the first reporter gene.

11. The method of claim 9 wherein a portion of the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites inverts the flanked portion of the first reporter gene which prevents expression of the first reporter gene.

12. The method of claim 5 wherein recombination between the third and fourth recombination sites allows the second reporter gene to be expressed.

13. The method of claim 12 wherein the second nucleic acid sequence further comprises a spacer sequence flanked by the third and fourth recombination sites, wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene is not expressed, wherein recombination of the third and fourth recombination sites excises the spacer sequence which allows the second reporter gene to be expressed.

14. The method of claim 13 wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene is not transcribed.

15. The method of claim 13 wherein the second reporter gene encodes a protein, wherein the spacer sequence interrupts the second reporter gene such that the protein encoded by the second reporter gene is not translated.

16. The method of claim 13 wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene produces an inactive expression product.

17. The method of claim 12 wherein a portion of the second reporter gene is inverted, wherein the inverted portion of the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites inverts the inverted portion of the second reporter gene which allows the second reporter gene to be expressed.

18. The method of claim 5 wherein recombination between the third and fourth recombination sites prevents expression of the second reporter gene to be expressed.

19. The method of claim 18 wherein the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites excises the second reporter gene which prevents expression of the second reporter gene.

20. The method of claim 18 wherein a portion of the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites inverts the flanked portion of the second reporter gene which prevents expression of the second reporter gene.

21. The method of claim 1 wherein the first nucleic acid sequence is a first nucleic acid construct and the second nucleic acid sequence is on a second nucleic acid construct.

22. The method of claim 21 wherein the first nucleic acid construct is an extrachromosomal vector and the second nucleic acid construct is in the genome of a host cell.

23. The method of claim 1 wherein the first and second nucleic acid constructs are on the same nucleic acid construct.

24. (Amended) A method for producing site-specific recombination of DNA, comprising,

contacting a variant recombinase identified by the method of claim 1 with third and fourth DNA sequences,

wherein the third DNA sequence comprises a fifth recombination site and the fourth DNA sequence comprises a sixth recombination site,

wherein the variant recombinase mediates recombination between the fifth and sixth recombination sites thereby producing the site specific recombination.

25. (Amended) The method of claim 24 wherein the fifth recombination site, the [second] sixth recombination site, or both, are variant recombination sites.

26. (Amended) The method of claim 24, wherein the third and fourth DNA sequences are connected by a pre-selected DNA segment.

27. (Amended) The method of claim 26, wherein the fifth and sixth recombination sites have the same orientation and the site-specific recombination of DNA is a deletion of the pre-selected DNA segment.

28. The method of claim 27, wherein the pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

29. (Amended) The method of claim 27 further comprising contacting the variant recombinase with a fifth DNA sequence comprising a seventh recombination site, wherein the fourth and fifth DNA sequences are connected by a second pre-selected DNA segment.

30. (Amended) The method of claim 29 wherein the fifth recombination site is a variant recombination site recognized by the variant recombinase and not by wild type recombinase, and wherein the sixth and seventh recombination sites are recombination sites recognized by wild type recombinase and by the variant recombinase.

31. (Amended) The method of claim 30 further comprising, prior to contacting the variant recombinase with the fifth, sixth, and seventh recombination sites, contacting the

recombination sites with wild type recombinase, thereby producing site specific recombination between the sixth and seventh recombination sites resulting in a deletion of the second pre-selected DNA segment.

32. The method of claim 29, wherein the second pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

33. (Amended) The method of claim 26, wherein the fifth and sixth recombination sites have opposite orientations and the site-specific recombination is an inversion of the nucleotide sequence of the pre-selected DNA segment.

34. (Amended) The method of claim 33, wherein the fifth and sixth recombination sites are variant recombination sites recognized by the variant recombinase.

35. The method of claim 33, wherein the pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

36. (Amended) The method of claim 24, wherein the fourth and fifth DNA sequences are introduced into two different DNA molecules and the site-specific recombination is a reciprocal exchange of DNA segments proximate to the recombination sites.

37. (Amended) The method of claim 36, wherein the fifth and sixth recombination sites are variant recombination sites recognized by the variant recombinase.

38. (Amended) The method of claim 24 wherein the fourth DNA sequence includes a label, wherein recombination between the fifth and sixth recombination sites associates the label with the third DNA sequence.

39. (Amended) The method of claim 38 wherein the third DNA sequence is a large circular DNA molecule.

40. The method of claim 24 wherein recombination occurs in a cell.

41. (Amended) The method of claim 40 wherein the variant recombinase is contacted with the third and fourth DNA sequences by introducing into the cell a sixth DNA sequence comprising DNA encoding the variant recombinase.

C6

42. (Amended) The method of claim 41, wherein the sixth DNA sequence further comprises a regulatory nucleotide sequence and expression of the variant recombinase is produced by activating the regulatory nucleotide sequence.

43. The method of claim 40, wherein the cell is a eukaryotic cell, a mammalian cell, a yeast cell, a fungal cell, a prokaryotic cell, a bacterial cell, an archae bacterial cell, or a cell in a multicellular organism.

44. The method of claim 43 wherein the multicellular organism is a plant, an animal, or a mammal.

45. (Amended) The method of claim 40, wherein the third and fourth DNA sequences are connected by a pre-selected DNA segment, wherein the first and second recombination sites have the same orientation and the site-specific recombination of DNA is a deletion of the pre-selected DNA segment.

C6

Marked Up Version of Amended Specification Paragraphs
Pursuant to 37 C.F.R. § 1.121(b)(1)(iii)

ATL1 #519277 v1